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(71) Applicant *(for all designated States except US)*: UNIVERSITY COLLEGE LONDON [GB/GB]; Gower Street, London WC1E 6BT (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: RADEMACHER, Thomas, William [US/GB]; Foxcombe, The Ridgeway, Boars Hill, Oxford OX1 5EY (GB). GREENBAUM, Leslie [GB/GB]; 4 Hunters Court, Friars Lane, Richmond, Surrey TW9 1NX (GB). McLEAN, Patricia [GB/GB]; 4 Hunters Court, Friars Lane, Richmond, Surrey TW9 1NX (GB).

(74) Agents: KIDDLE, Simon, J. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

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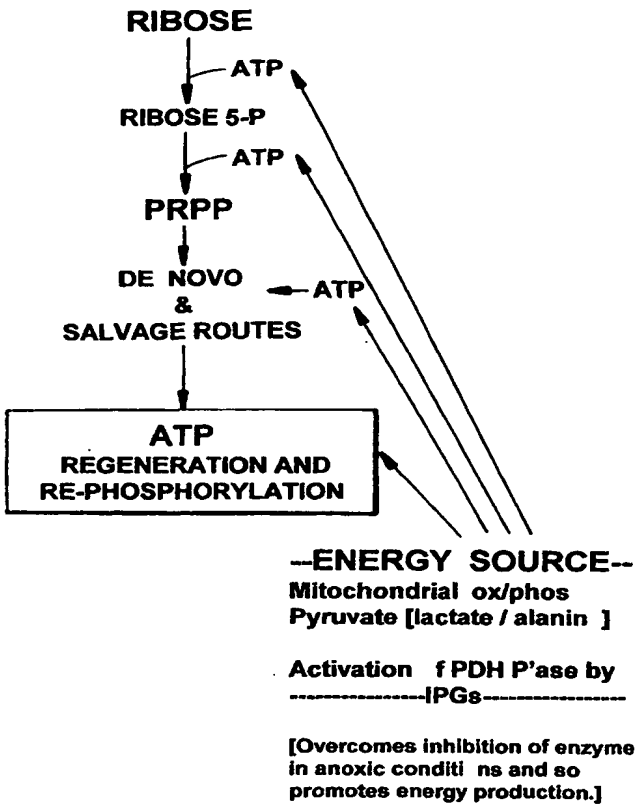
(57) Abstract

Compositions comprising inositolphosphoglycans (IPGs) and ribose are disclosed, and their use in the prevention or treatment of ischaemic-reperfusion injury. This treatment increases the energy generating systems of cells by employing the mitochondrial oxidative restoration system. The use of the compositions in preserving organs for transplantation is also disclosed.



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INOSITOLPHOSPHOGLYCAN AND RIBOSE FOR TREATMENT OF ISCHAEMIA-REPERFUSION INJURY

Field of the Invention

The present invention relates to material and methods relating to the prevention or treatment of ischaemia-reperfusion injury, and in particular to compositions comprising inositolphosphoglycans (IPGs) and their medical use in the prevention or treatment of ischaemia.

Background of the Invention

The search for novel therapies for ischaemic-reperfusion injury in the heart has been a subject of intense research, both for recovery from open-heart surgery, where the limited capacity for the heart to survive ischaemia is a well researched problem (Stanley et al, 1997), and from the viewpoint of modulating the extent of damage incurred during episodes of cardiac ischaemia (Stanley et al, 1997). It is also well established that the incidence of coronary heart disease is a major factor in the morbidity and mortality of diabetic patients (Fuller et al, 1983; Hillier et al, 1988). There is also evidence that standard drugs for the treatment of diabetes of the sulphonylurea group may have negative effects, including those on K^+ channel function (Smits & Thien, 1995; Muhlhauser et al, 1997).

The complexity of the events following ischaemia-reperfusion is such that there is a very wide ranging database of potential therapeutic and cardioplegic agents targeting differing aspects of the cascade leading to damage to cardiac function. It has been apparent from

work as early as the 1960s (Danforth et al, 1960; Berne, 1963) to the present (Zimmer, 1996; Houston et al, 1997) that a key feature of the cascade of interlinked biochemical events following ischaemic-reperfusion injury centres on the loss of adenine nucleotides from the myocardium. There is, thus, an absolute requirement for the restitution of the intracellular ATP concentration and the energy charge of the cell in order to restore normal cardiac function.

Adenine nucleotide synthesis can occur via utilization or reutilisation of adenine nucleotide breakdown products via the salvage pathway, or via *de novo* synthesis from small molecular weight precursors. The former is the most effective in terms of energy requirement (Mangano, 1997; Meldrum et al, 1997).

However, in addition to the requirement for the purine ring, a supply of phosphoribosylpyrophosphate (PRPP) is essential both for the salvage and *de novo* routes of synthesis; this latter compound is, in turn, subject to tight regulation and is dependent upon a supply of ribose-5-phosphate (Kunjara et al, 1987). Zimmer (1980) demonstrated that restitution of myocardial adenine nucleotides was accelerated by ribose, as was the normalisation of depressed heart function in rats (Zimmer, 1983). This author stated that "The advantage of ribose over other metabolic interventions is that is does not affect the haemodynamics of the heart with an ultimate change in oxygen demand and that is has no vasoactive properties which may result in afterload

alterations".

Recently, Zimmer (1996) reported that in two *in vivo* rat models, the overloaded and catecholamine-stimulated heart and the infarcted heart, the normalisation of the cardiac adenine nucleotide pool by ribose was accompanied by improvement in global heart function. Further, the combined treatment with ribose and adenine or inosine in isoproteronal-treated rats was more effective in the restoration and completely restored the ATP level within a shorter period of time than either treatment alone.

Summary of the Invention

While the results showing the effect of repletion of cardiac ATP are encouraging, the prior art approaches described above suffer from the disadvantage that the biosynthetic pathways themselves require ATP, as does the reconversion of AMP to ADP and ATP, the required ATP being the very compound in short supply. Further, as mentioned above, the complexity of the biochemistry associated with ischaemia means that it is not clear from the prior art how alternative approaches could avoid this problem.

The present invention relates to the finding that inositolphosphoglycans (IPGs), and in particular P-type IPGs, or their synthetic analogues, can be used to generate ATP from ADP while helping to avoid the production of toxic byproducts and helping to minimise the ATP requirement for the process. Thus, compositions comprising IPGs can be used to prevent or treat

ischaemia-reperfusion, in particular in conditions where there is a reduction or risk of reduction in cellular ATP levels, e.g. in cardiac ischaemia, in surgery (especially heart or transplant surgery), in preserving organs for transplantation, in the treatment of stroke and as an anti-apoptosis agent to protect against cell death (especially in muscle cells).

Accordingly, in a first aspect, the present invention provides a composition for treating an ischaemic-reperfusion injury, the composition comprising an inositolphosphoglycan (IPG) or an IPG synthetic analogue, and ribose

In a further aspect, the present invention provides the use of an inositolphosphoglycan (IPG) for the preparation of a medicament for the treatment of ischaemic-reperfusion injury.

The IPGs present in the medicament can be P- or A-type IPGs, or synthetic analogues of them. The production of IPGs and IPG analogues is discussed further below. Preferably, the IPG is a P-type IPG or a P-type synthetic analogue.

The present invention is based on the realisation that an alternative approach to the problem of increasing the energy generating systems of the cell is to employ the mitochondrial oxidative restoration system, in particular by the regulation of the key enzyme for the entry of pyruvate into the tricarboxylic acid cycle, pyruvate

dehydrogenase. Accordingly, the present proposal centres upon the use of naturally occurring activators of pyruvate dehydrogenase phosphatase, the inositolphosphoglycans, to promote the conversion of pyruvate dehydrogenase to the active form, thereby enhancing the rephosphorylation of AMP and ADP.

Advantageously, the composition includes one or more other components, in combination with the IPGs, for use in the treatment of ischaemia-reperfusion injury as described herein. Among the agents to be used in combination with IPGs from different sources are:

(1) Adenosine and purine compounds as precursors of ATP and as modulators of TNFα action (see Bouchard & Lamontagne, 1998; de Jong et al, 1997; Meldrum et al, 1997).

(2) Ribose as a precursor of PRPP (see Kunjara et al, 1987; Zimmer, 1996).

(3) Nicotinamide and derivatives to prevent the loss of NAD and ATP by inhibition of poly-ADP ribose synthetase (see Bromme & Holz, 1996; Zingarelli et al, 1996; Gilad et al, 1997; Thiememann et al, 1997).

(4) Ca^{2+} uptake inhibitors (see Ferrari et al, 1996; Loh et al, 1998; Russ et al, 1996).

(5) Addition of IPGs to established cardioplegic solutions (see Choong and Gavin, 1996; Bozkurt et al,

1997).

(6) Maintenance of glutathione systems (see Konorev et al, 1996). Glutathione in its reduced form (GSH) is an important factor in the prevention of damage by hydrogen peroxide. Hydrogen peroxide is a component of ischaemia-reperfusion injury and protection is afforded by the action of glutathione peroxidase and GSH. The importance of GSH and the pentose phosphate pathway in the chain reactions protecting the cell from free radical damage is illustrated in Figure 1 from Zubairu et al, 1983.

(7) Endothelin inhibitors (see Goodwin et al, 1997; Pernow & Wang, 1997). Endothelin-1 (ET-1) is an extremely potent vasoconstrictor peptide derived from vascular endothelial cells. During and following myocardial ischaemia and reperfusion, the myocardial production and release of ET-1 is stimulated and the coronary constriction to ET-1 is enhanced. The pathophysiological role for ET-1 in the development of ischaemia has a strong basis and the potential for cardioprotective effects of ET-1 antagonists has been considered by Pernow and Wang (1997).

Ischaemia-reperfusion injury can arise in a wide range of conditions and the medicament can be used to treat these conditions. Examples include ischaemia resulting from myocardial infarct, during surgery (especially open heart surgery, or during organ transplantation, e.g. employing the medicament as a cardioplegia solution for heart or lung bypass surgery), and in stroke. The medicament can

also be used to ameliorate the effects of ischaemia in tissues, in particular as an anti-apoptotic agent to prevent cell death following ischaemia, e.g. muscle cell death.

In a further aspect, the present invention provides a method for preserving an organ for transplantation, the method comprising exposing the organ with a composition comprising an inositolphosphoglycan (IPG), and optionally one or more of the components mentioned above. As ischaemia is common in organs for transplantation, this approach is useful for preserving the energy level present in the organ prior to transplantation and during surgery. Conveniently, the composition can be perfused through the organ or used to store the organ prior to transplantation, i.e. be a storage medium for the organ.

In a further aspect, the present invention provides compositions comprising a P-type IPG and ribose. In these compositions, the IPG drives mitochondrial oxidation and results in ATP generation from ADP without production of toxic byproducts. Preferably, the composition additionally comprises a purine or purine nucleotide precursor to provide the basic structural element of ATP. Other possible components of the composition are described above.

This composition is useful in organ preservation, in general surgery (e.g. as a perfusion fluid) and in other situations for the prevention or treatment of ischaemia in cells. Preferably, the composition is supplied as a

powder or concentrate from which a liquid composition can be prepared. Alternatively, the composition can be supplied ready to use in as a liquid. Formulations and optional ingredients of the composition are discussed further below.

In further aspects, the present invention provides above compositions for use in a method of medical treatment, for example in the preparation of a medicament for the treatment of ischaemic conditions discussed above.

Embodiments of the present invention will now be described by way of example and not by limitation with reference to the accompanying drawings.

Brief Description of the Drawings

Figure 1 shows the correlation between the hepatic PRPP concentration and the log of ribose 5-phosphate and the flux through the oxidative pentose phosphate assay pathway (C1-C6) in different dietary and hormonal conditions in rats.

Figure 2 shows the correlation between the hepatic PRPP concentration and ATP and energy charge (EC), free cytosolic NAD^+/NADH and NAD^+/NADPH in different dietary and hormonal conditions in rats.

Figure 3 shows the correlation between the hepatic PRPP concentration and ADP, AMP and Pi in different dietary and hormonal conditions in rats.

Figures 4A and 4B shows the steady state concentration and the effect of insulin on extractable IPG A-type from the heart and other tissues from adult male rats. Figure 4A shows the results of a lipogenesis assay and figure 4B shows a cAMP-dependent protein kinase A assay. The solid columns show results in the absence of insulin, while the hatched columns show results 2 minutes after injection with insulin. 1 unit is the amount of A-type IPG causing a 50% increase in the basal rate of lipogenesis or a 50% decrease in the activity of cAMP dependent protein kinase.

Figures 4C and 4D show the steady state concentration and the effect of insulin on extractable IPG P-type from heart and other tissues from adult male rats. Figure 4C shows a PDH phosphatase assay and figure 4D shows a cAMP-dependent protein kinase-P assay. The solid columns show results in the absence of insulin, while the hatched columns show results 2 minutes after injection with insulin. 1 unit is the amount of P-type IPG causing a 50% increase in the activity of PDH phosphatase or a 50% decrease in the activity of cAMP dependent protein kinase.

Figures 4E and 4F show the results of a thymidine incorporation into EGF receptor transfected 3T3 cells, plotted against IPG A-type and IPG P-type concentrations respectively.

Figure 5 shows a schematic setting out the role of ribose, IPGs and selected substrates on the prevention or

recovery from ischaemic damage according to the present invention.

Figure 6 shows a schematic setting out the site of action of IPG P-type in the activation of the PDH complex.

Detailed Description of the Invention

IPGs and IPG Analogues

Studies have shown that A-type mediators modulate the activity of a number of insulin-dependent enzymes such as cAMP dependent protein kinase (inhibits), adenylate cyclase (inhibits) and cAMP phospho-diesterases (stimulates). In contrast, P-type mediators modulate the activity of insulin-dependent enzymes such as pyruvate dehydrogenase phosphatase (stimulates), glycogen synthase phosphatase (stimulates) and cAMP dependent kinase (inhibits). The A-type mediators mimic the lipogenic activity of insulin on adipocytes, whereas the P-type mediators mimic the glycogenic activity of insulin on muscle. Both A-and P-type mediators are mitogenic when added to fibroblasts in serum free media. The ability of the mediators to stimulate fibroblast proliferation is enhanced if the cells are transfected with the EGF-receptor. A-type mediators can stimulate cell proliferation in the chick cochleovestibular ganglia.

Soluble IPG fractions having A-type and P-type activity have been obtained from a variety of animal tissues including rat tissues (liver, kidney, muscle brain, adipose, heart) and bovine liver. A- and P-type IPG biological activity has also been detected in human liver

and placenta, malaria parasitized RBC and mycobacteria. The ability of an anti-inositolglycan antibody to inhibit insulin action on human placental cytotrophoblasts and BC3H1 myocytes or bovine-derived IPG action on rat diaphragm and chick cochleovestibular ganglia suggests cross-species conservation of many structural features. However, it is important to note that although the prior art includes these reports of A- and P-type IPG activity in some biological fractions, the purification or characterisation of the agents responsible for the activity is not disclosed.

A-type substances are cyclitol-containing carbohydrates, also containing Zn^{2+} ion and optionally phosphate and having the properties of regulating lipogenic activity and inhibiting cAMP dependent protein kinase. They may also inhibit adenylate cyclase, be mitogenic when added to EGF-transfected fibroblasts in serum free medium, and stimulate lipogenesis in adipocytes.

P-type substances are cyclitol-containing carbohydrates, also containing Mn^{2+} and/or Zn^{2+} ions and optionally phosphate and having the properties of regulating glycogen metabolism and activating pyruvate dehydrogenase phosphatase. They may also stimulate the activity of glycogen synthase phosphatase, be mitogenic when added to fibroblasts in serum free medium, and stimulate pyruvate dehydrogenase phosphatase.

Methods for obtaining A-type and P-type IPGs are set out in Caro et al, 1997 and in WO98/11116 or WO98/11117. The

present invention can employ IPGs found in nature, for instance in tissues such a liver or placenta from animals such as human, pig, rat or other animals), and obtained using methods described in the above applications. These IPGs are preferably purified from the tissues, and more preferably purified to homogeneity. As defined herein, "substantially purified" describes IPGs which have been separated from components which are naturally present with the IPGs in the source tissue. Preferably, the compositions are at least 75%, more preferably at least 90%, more preferably at least 95%, and still more preferably at least 99% by weight of IPGs.

Alternatively or additionally, the present invention can employ cyclitol-containing IPG analogues, e.g. inositol-containing IPG analogues. These compounds have the advantage that they can be more readily prepared using synthetic organic chemistry methods, rather than being extracted from natural source materials. Preferred P-type synthetic analogues contain chiro-inositol, or a derivative thereof, as a structural unit or motif, and have one or more of the properties of P-type IPGs indicated above, especially activation of pyruvate dehydrogenase phosphatase. An example of a chiro-inositol containing IPG analogue is compound C4, 1D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-chiro-inositol 1-phosphate which can be synthesised as described in Jaramillo et al, 1994.

Preferred A-type synthetic analogues contain myo-inositol, or a derivative thereof, as a structural unit

or motif and have one or more of the properties of A-type IPGs indicated above. An example of a myo-inositol containing IPG analogue is compound C3 1D-6-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-myo-inositol 1,2-(cyclic phosphate), which can be been prepared as described in Zapata et al, 1994.

Pharmaceutical Compositions

The compositions of the invention can be formulated according to the specific application which the composition is intended to treat. The compositions may comprise, in addition to the one or more IPGs, and optionally one or more of the above components, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient(s). The precise nature of the carrier or other material may depend on the route of administration, e.g. intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes. For embodiments in which the medicaments or compositions of the invention are used in organ preservation, they can be formulated so that they are suitable for storing or perfusing organs or tissue.

The compositions may be supplied in the form of a powder or concentrate from which a composition can be prepared. Alternatively, the composition may be supplied in a ready to use form, e.g. as a liquid. In either event, the composition may include other active ingredients,

adjuvants or carriers. Thus, physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

In embodiments in which the composition is used in the prophylactic or therapeutic treatment of conditions associated with a risk of ischaemia, preferably the composition is administered to a patient via intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction. In this case, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as sodium chloride injection, Ringer's injection, lactated Ringer's injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required. Injection is a preferred mode of delivery for compositions for treating ischaemia that results from myocardial infarction, stroke or to treat or protect against apoptosis.

The active ingredients in the composition are preferable administered to an individual in preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of

administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Experimental

Experiments in this laboratory have shown with rat heart preparations that the tissue PRPP concentration in anoxic conditions fell and was partially restored by addition of ribose to the medium. Perhaps of greater significance was our observation of the decline in cellular PRPP in a range of tissues, including heart, in experimental diabetes (see Table 1). These data suggest that ribose or a ribose precursor and/or purine derivatives could advantageously be included in the medicaments compositions of the invention.

While reported effects of repletion of cardiac ATP are encouraging, it is apparent that these biosynthetic processes themselves require ATP, as does the

reconversion of AMP to ADP and ATP, the required ATP being the very compound in short supply. Thus, any mechanism increasing the energy generating systems of the cell, primarily and most effectively via the mitochondrial oxidative restoration, would be advantageous to the process of cellular restoration. In this context, the regulation of the key enzyme for the entry of pyruvate into the tricarboxylic acid cycle, the pyruvate dehydrogenase complex, must be considered.

This enzyme is highly regulated by, among other factors, the energy status of the cell, by the NADH/NAD+ ratio and by the acetyl CoA/CoA ratio, via the interconversion of active/inactive forms of pyruvate dehydrogenase by phosphorylation/dephosphorylation reactions regulated by pyruvate dehydrogenase kinase and regulation of this enzyme complex at the pyruvate crossroads. This system operates in a manner such that ischaemic conditions activate PDH kinase dehydrogenase and so shut off energy production at this step. In order to circumvent this inhibition, even in ischaemia, it is necessary to activate the PDH phosphatase and this can be accomplished by the presence of IPGs. Pyruvate dehydrogenase activity is the most important determinant of whether pyruvate is converted to lactate, leading to lactic acidosis and a low level of ATP from glycolysis, or whether the highly efficient ATP generating system of the tricarboxylic acid cycle will be facilitated.

The present invention centres upon the use of naturally occurring activators of pyruvate dehydrogenase

phosphatase, the inositolphosphoglycans, to promote the conversion of pyruvate dehydrogenase to the active form (Rademacher et al, 1994; Varela-Nieto et al, 1998), thereby enhancing the rephosphorylation of AMP and ADP. The preferred combination of purine nucleotide precursors (to provide the basic structural element of the required ATP), together with ribose (to provide the ribose 5-phosphate for PRPP formation) and inositolphosphoglycans (to shift the pyruvate dehydrogenase complex towards the active form, generate energy and decrease lactic acidosis) can be used to treat ischaemic conditions, e.g. ischaemic heart conditions, and the loss of ATP. As can be seen from Figure 5, such a therapy would supply all three major elements required for the restoration of the energy charge of the cell.

(1) Ribose, as the precursor of the synthesis of the adenine lost from the cell during extended ischaemia;

(2) PRPP, an essential component of the adenine biosynthetic pathway; and,

(3) An increase energy yield from carbohydrate fuel which can provide the energy needed for biosynthetic processes in (1) and (2) and also to rephosphorylate such ADP and AMP as remains in the cell to ATP.

Therefore, the approach of using inositolphosphoglycans either alone or together with other precursors of adenine nucleotide synthesis and compounds protecting against loss of ATP (e.g. by inhibition of poly ADP ribose), in

the treatment of ischaemic conditions in heart, kidney, brain or other organs, is a fundamental new approach to attempting to limit cell damage. In a preferred embodiment of the invention, the combination of ribose, purine precursors and nicotinamide, the latter to prevent lost of NAD and ATP by inhibition of polyADP ribose synthase, with the inositolphosphoglycans, the potent second messenger system functioning in the regulation of protein phosphorylation/dephosphorylation cycles, is a multifaceted attack on the very basis of cellular damage in ischaemic conditions, that is the loss of ATP.

Table 1 demonstrates that in diabetes, there is a drop in tissue levels of PRPP. This drop could make diabetic patients more at risk of morbidity following an ischaemic attack. It is well established that both the incidence and complications of coronary heart disease are elevated in diabetic patients and decreased tissue levels of PRPP could be the crucial link. Thus, the present invention is particularly suited to the treatment of ischaemic conditions arising from diabetes. Figure 1 demonstrates that tissue levels of ribose 5-phosphate are important in maintaining PRPP levels and Figure 5 shows that ribose is the direct precursor of ribose 5-phosphate. Therefore, one important component in maintaining high levels of PRPP is to provide ribose as the precursor for ribose 5-phosphate.

Figures 2 and 3 demonstrate that in order to have high levels of PRPP in tissues, the cellular energy charge must be high. Under anoxic conditions, this is difficult

since the enzyme PDH kinase is activated. The action of this enzyme is to inactivate the PDH complex, which is involved in the biosynthesis of acetyl-CoA and NADH. The NADH so generated in the reperfusion period is oxidized by the electron transport chain to generate ATP. The acetyl-CoA is a substrate for the Krebs cycle in which one glucose can be oxidized to 36 ATPs via the generation of further NADH. The action of IPG-P type mediators is to activate PDH phosphatase which counteracts the PDH kinase and allows for activation of the PDH complex. This activation is shown in Figure 6. The action of the IPG-P type and the amounts recovered from various tissues before and after insulin infusion are shown in Figure 4C and D. In particular, an increase in activity is found in muscle and kidney upon insulin infusion. In contrast, decreased activity is found in heart, adipose tissue and brain (Figure 4C). These data demonstrate that an insulin infusion could not substitute for a direct infusion of the IPG-P type. Figure 5 shows that an insulin infusion will also affect the IPG-A activity differentially in tissues and this effect would not occur on infusion of just IPG-P compound or its analogues.

TABLE 1. EFFECTS OF EXPERIMENTAL DIABETES ON PHOSPHORIBOSYL PYROPHOSPHATE (PRPP) CONTENT OF HEART AND OTHER TISSUES

	PHOSPHORIBOSYL PYROPHOSPHATE CONTENT (nmoles/g tissue)		
Tissue	Control	STZ Diabetic (14 Days)	"P"
Heart	3.61±0.11 (15)	2.60±0.20 (6)	<0.01
Liver	10.5±0.64 (17)	7.60±0.43 (5)	<0.001
Lung	5.40±0.05 (16)	3.44±0.39 (5)	<0.001
Testis	5.0±0.30 (20)	2.5±0.9 (5)	<0.02
Blood glucose (mM)	7.0±0.45 (25)	28±3.0 (7)	<0.001
Body weight (g)	309±17 (20)	226±21 (7)	<0.01

The tissues were freeze-clamped and the PRPP content estimated as described by Kunjara et al (1987). The values are given as means ±SEM; Fisher's P values are given. The adult male rats were used 14 days after the induction of diabetes with streptozotocin.

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The following references are all expressly incorporated by reference.

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Claims:

1. A composition comprising an inositolphosphoglycan (IPG) or an IPG synthetic analogue and ribose.

2. The composition of claim 1 wherein the IPG is a P-type IPG.

3. The composition of claim 1 wherein the synthetic analogue is a P-type IPG synthetic analogue.

4. The composition of any one of the preceding claims, further comprising adenosine or purine, or a nucleotide precursor thereof.

5. The composition of claim 1 or claim 2, wherein the composition is a liquid composition.

6. The composition of claim 1 or claim 2, wherein the composition is a powder or concentrate from which a liquid composition can be prepared.

7. A composition of any one the preceding claims for use in a method of medical treatment.

8. Use of an inositolphosphoglycan (IPG) or an IPG synthetic analogue for the preparation of a medicament for the treatment of an ischaemic-reperfusion injury.

9. The use of claim 8 wherein the IPG is a P-type IPG.

10. The use of claim 8 wherein the synthetic analogue is a P-type IPG synthetic analogue.

11. The use of any one of claims 8 to 10, wherein the ischaemic-reperfusion injury arises from myocardial infarct, surgery or stroke.

12. The use of claim 11, wherein the surgery is open heart surgery, organ transplantation surgery, or heart or lung bypass surgery

13. The use of any one of claims 8 to 12, wherein the medicament is for the prevention of apoptosis following an ischaemic-reperfusion injury.

14. The use of any one of claims 8 to 13 wherein the medicament further comprises one or more of:

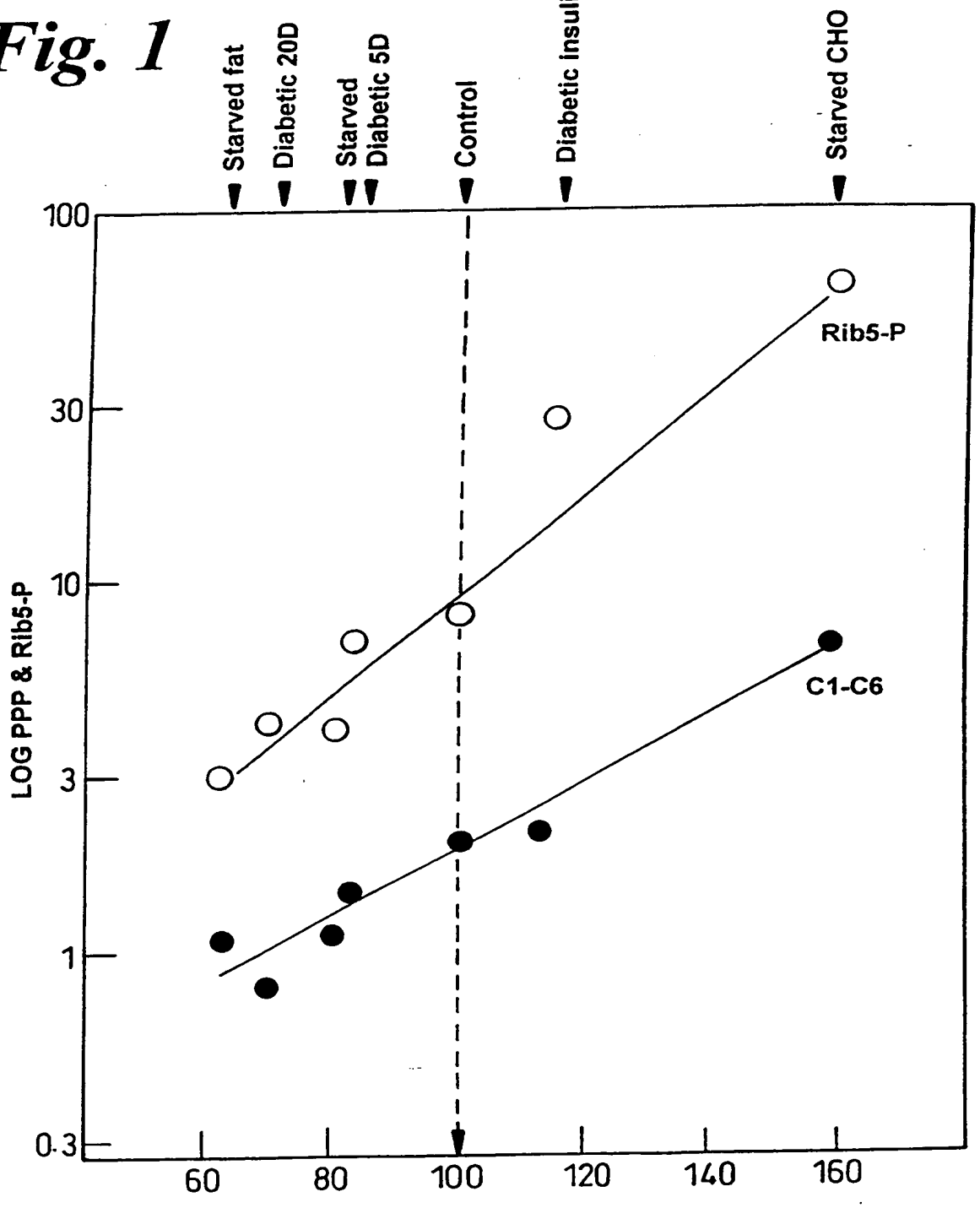
(a) adenosine or purine or a precursor thereof;
(b) ribose;
(c) nicotinamide or derivatives thereof;
(d) a Ca^{2+} ion uptake inhibitor;
(e) a cardioplegic solution;
(f) means to maintain the glutathione system, such as glutathione peroxidase and the reduced form of glutathione (GSH); or,
(g) an endothelin inhibitor.

15. An *in vitro* method for preserving an organ for transplantation, the method comprising contacting the organ with a composition of any one of claims 1 to 7.

16. The method of claim 15 wherein the composition is perfused through the organ.

17. The method of claim 15 wherein the organ is stored in the composition prior to transplantation.

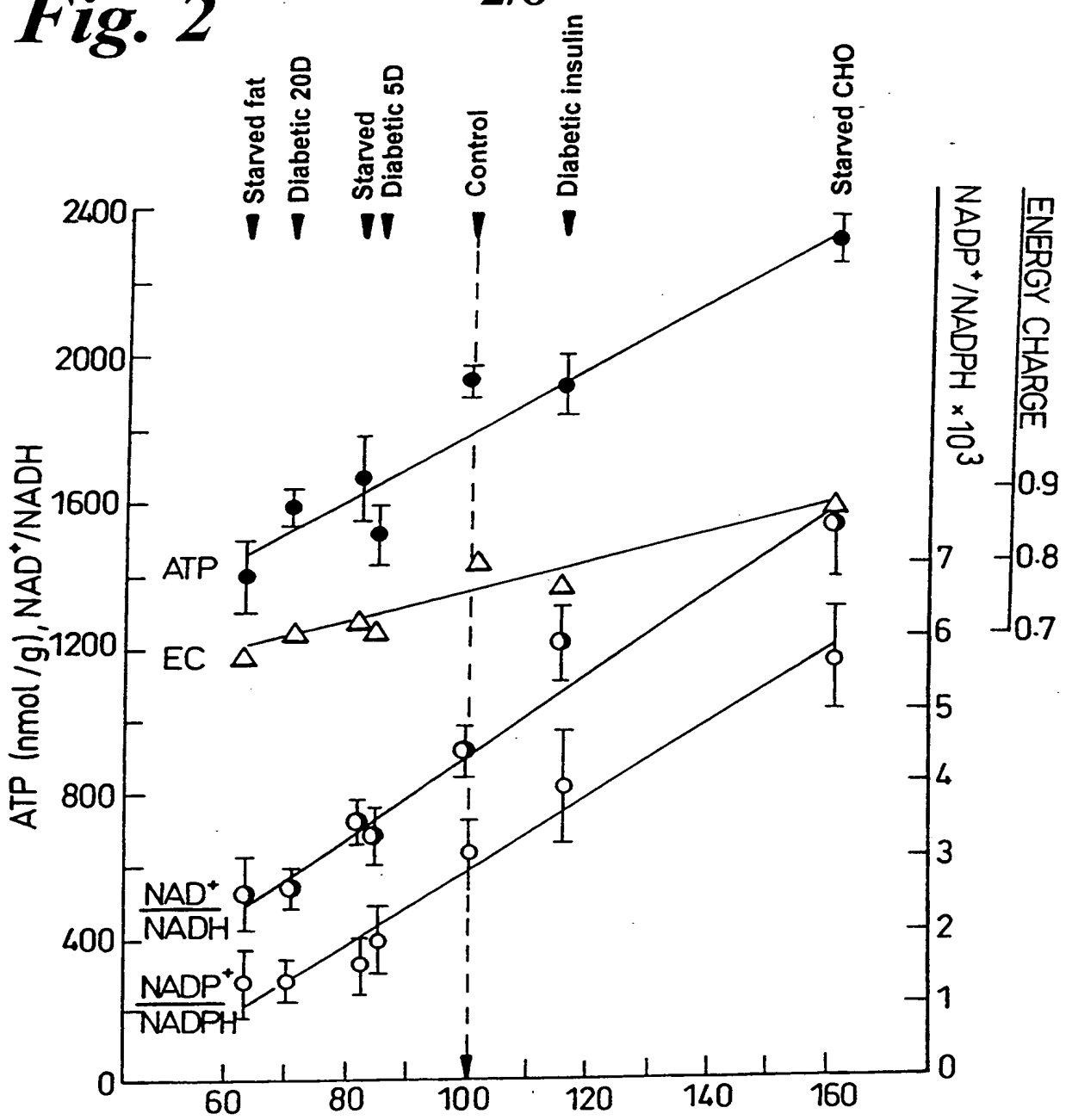
Fig. 1



PRPP CONTENT OF LIVER

CONTROL FED RAT LIVER = 100 %

Fig. 2



PRPP CONTENT OF LIVER

CONTROL FED RAT LIVER = 100%

Fig. 3

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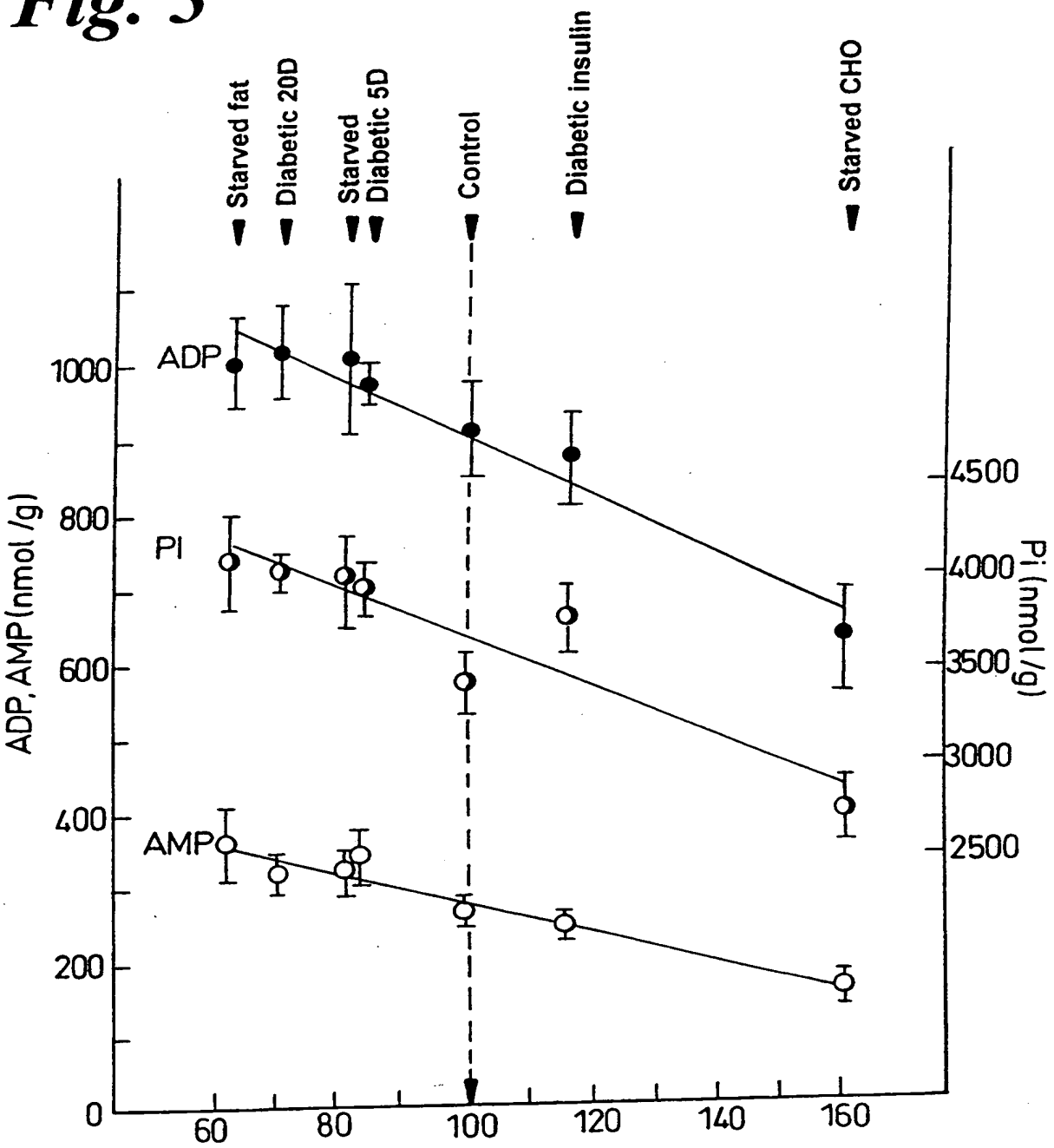


PRPP CONTENT OF LIVER

CONTROL FED RAT LIVER = 100%

Fig. 4 (A) **Lipogenesis. Adipocyte assay.**

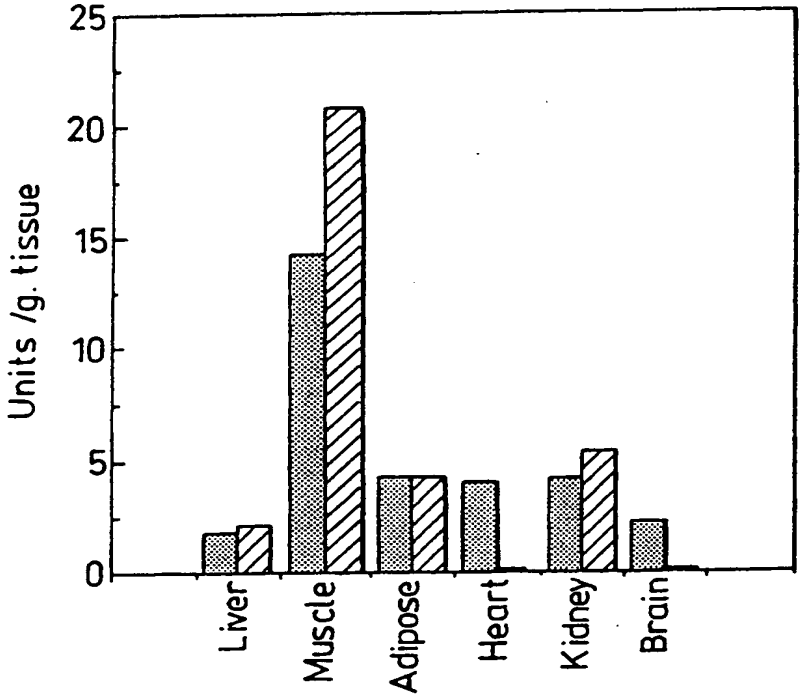


Fig. 4 (B) **cAMP-dependent protein kinase-A assay**

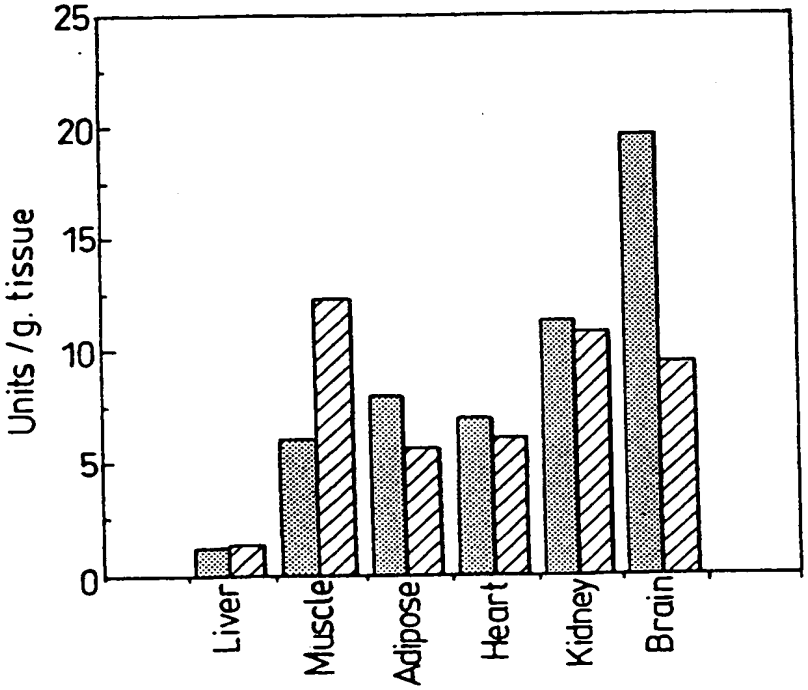


Fig. 4 (C) **PDH phosphatase assay**

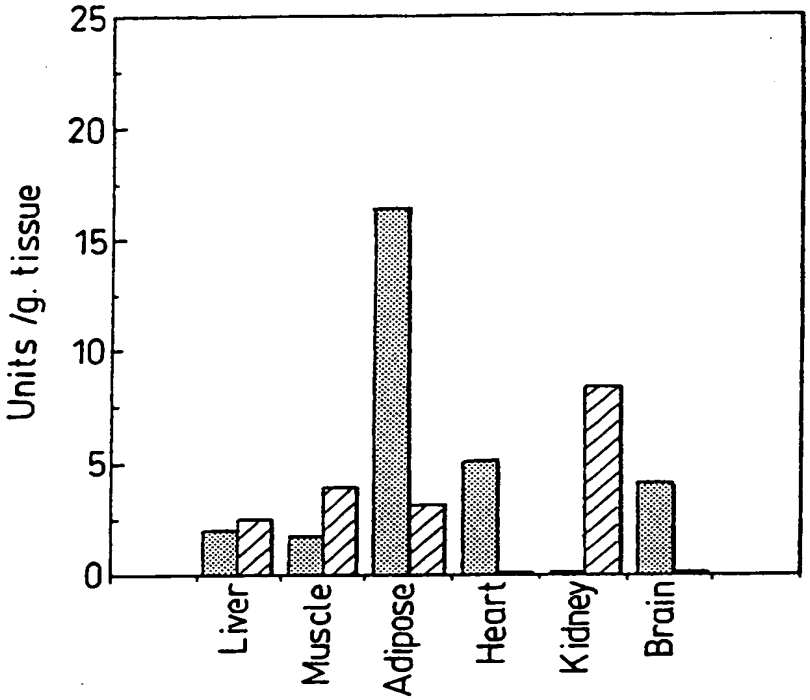
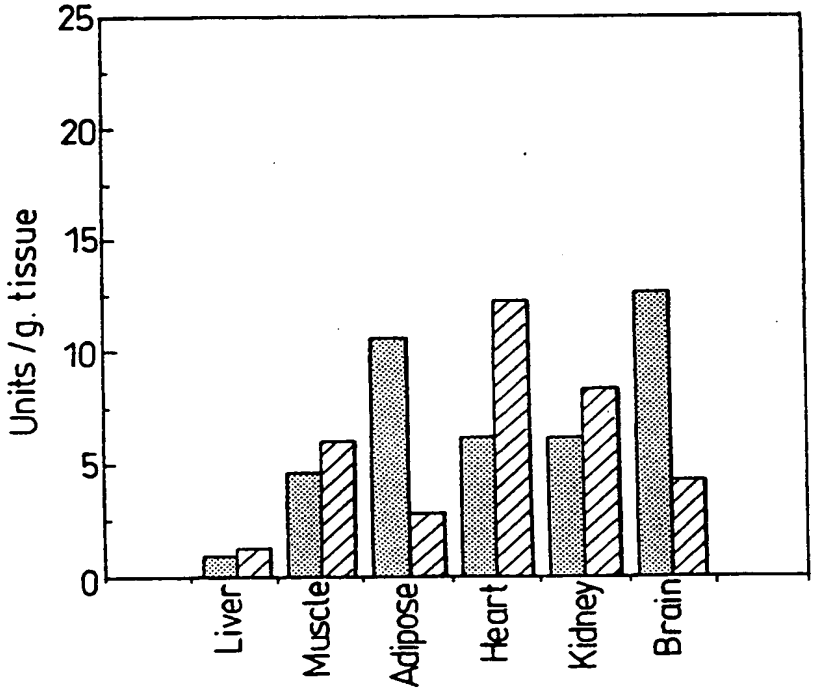


Fig. 4 (D) **cAMP-dependent protein kinase-P assay**



6/8

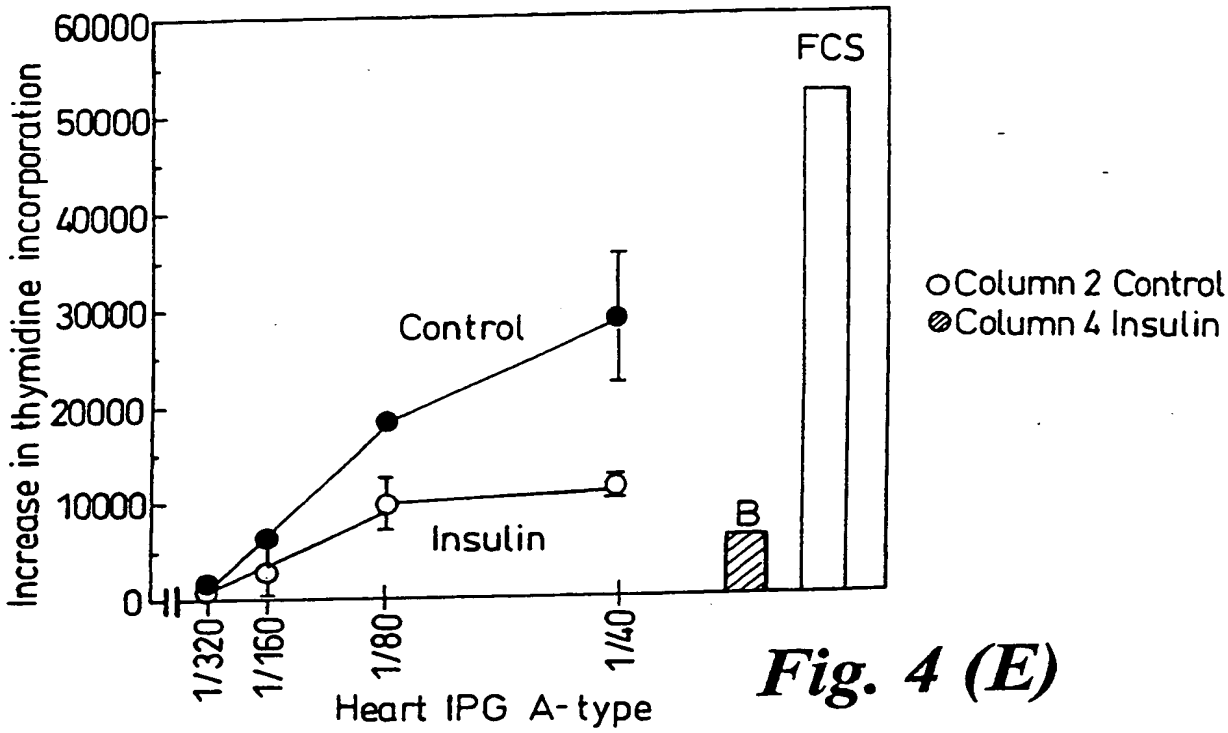


Fig. 4 (E)

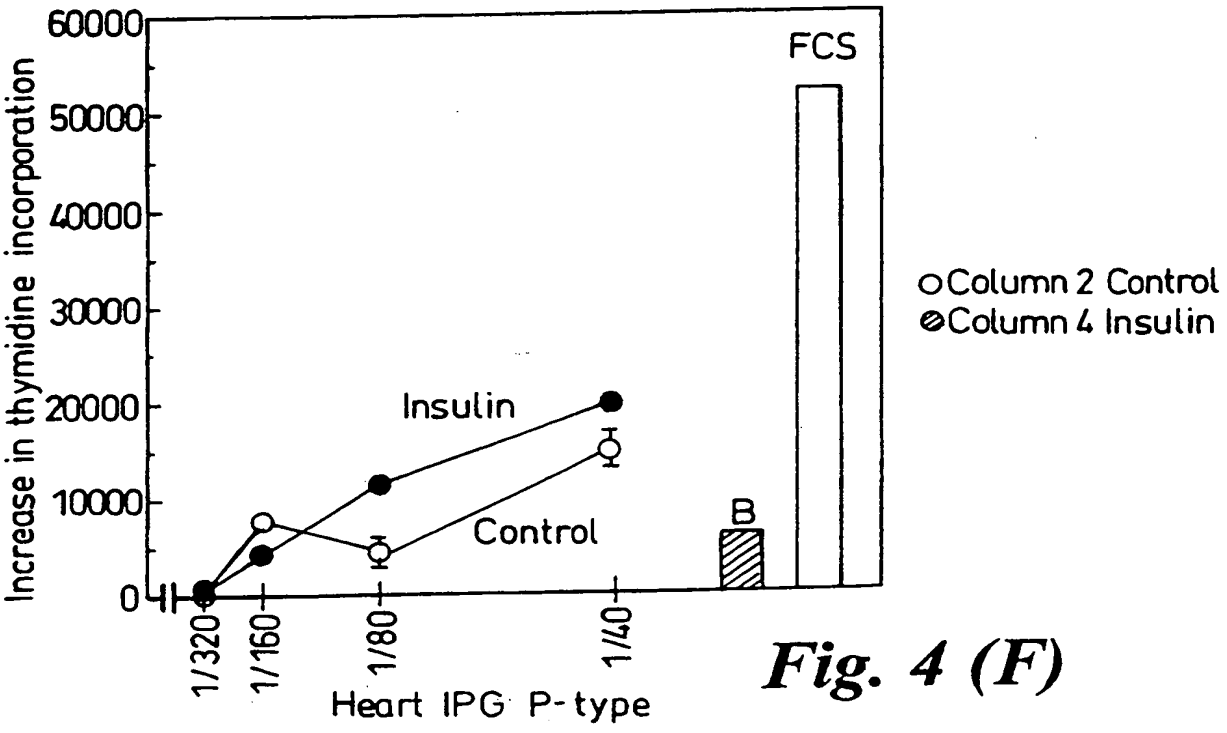


Fig. 4 (F)

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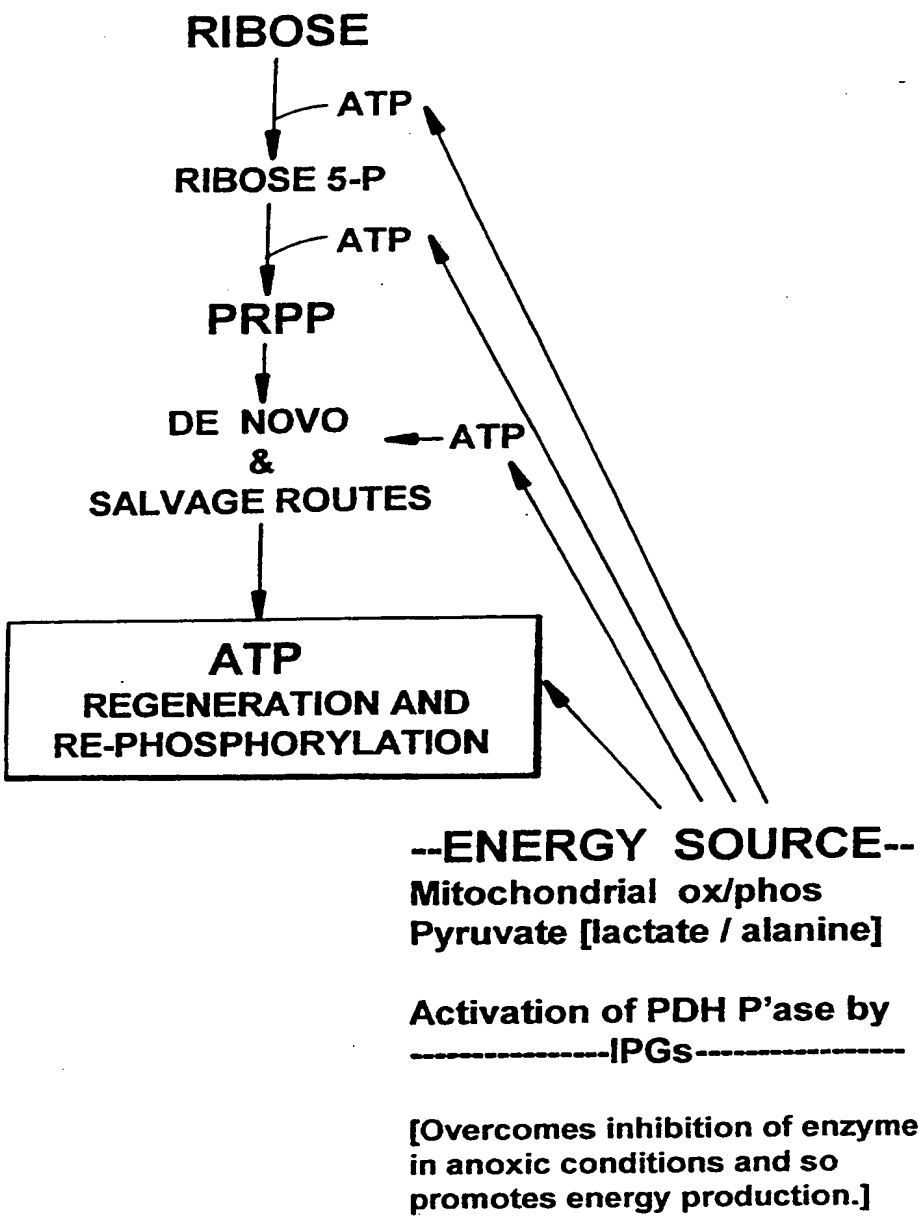
RIBOSE
ATP
RIBOSE 5-P
ATP
PRPP
DE NOVO
&
SALVAGE ROUTES
ATP
ATP
REGENERATION AND
RE-PHOSPHORYLATION
--ENERGY SOURCE--
Mitochondrial ox/phos
Pyruvate [lactate / alanine]
Activation of PDH P'ase by
----------IPGs----------
[Overcomes inhibition of enzyme
in anoxic conditions and so
promotes energy production.]

Fig 5

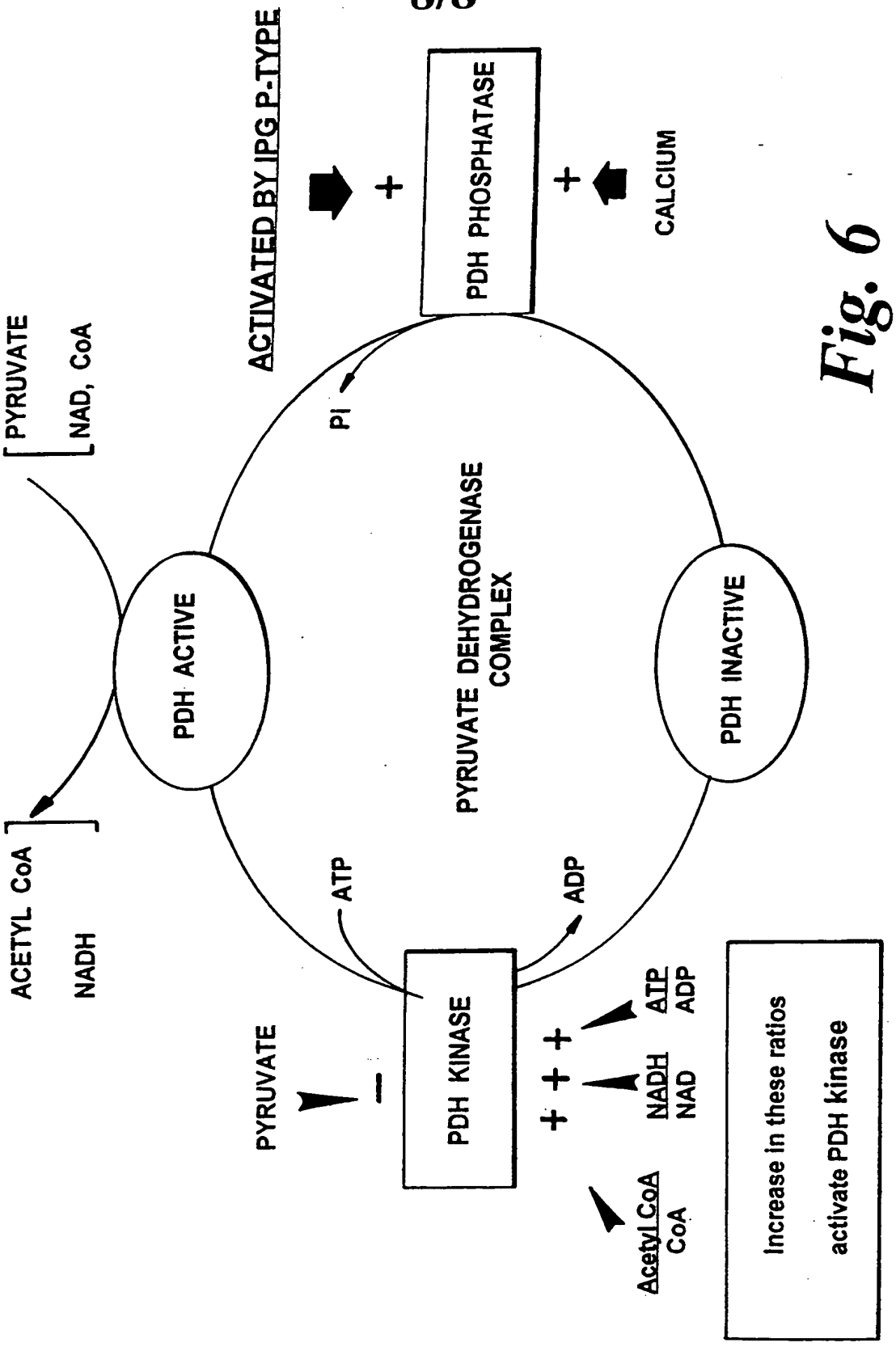
ACTIVATED BY IPG P-TYPE
PDH PHOSPHATASE
CALCIUM
PYRUVATE
NAD, CoA
PI
PDH ACTIVE
PYRUVATE DEHYDROGENASE COMPLEX
PDH INACTIVE
ACETYL CoA
NADH
ATP
ADP
PYRUVATE
PDH KINASE
ATP
ADP
NADH
NAD
Acetyl CoA
CoA
Increase in these ratios
activate PDH kinase

Fig. 6

TENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
SJK/BP5767348		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 01499	12/05/1999	29/06/1998
Applicant		
UNIVERSITY COLLEGE LONDON. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

INOSITOLPHOSPHOGLYCAN AND RIBOSE FOR TREATMENT OF ISCHAEMIA-REPERFUSION INJURY

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 5

[ ] as suggested by the applicant.

[X] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/GB 99/01499

Box I Observations where c rtain claims were found unsearchable (C ntinuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 8-17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Int. Application No
PCT/GB 99/01499

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9747192 A	18-12-1997	AU 6141296 A	07-01-1998
EP 0324227 A	19-07-1989	US 4871718 A	03-10-1989
		AT 77557 T	15-07-1992
		AU 600139 B	02-08-1990
		AU 1769088 A	29-06-1989
		CA 1325593 A	28-12-1993
		DE 3872372 A	30-07-1992
		DK 726088 A	30-06-1989
		ES 2045141 T	16-01-1994
		GR 90300016 T	07-06-1991
		IE 62263 B	11-01-1995
		JP 1175939 A	12-07-1989
		JP 1813299 C	27-12-1993
		JP 5020414 B	19-03-1993
		US 4923851 A	08-05-1990
		US 5391550 A	21-02-1995
EP 0652012 A	10-05-1995	CA 2103399 A	19-05-1995
EP 0845475 A	03-06-1998	DE 19649350 A	04-06-1998
		AU 4538297 A	04-06-1998
		CA 2222103 A	28-05-1998
		CN 1184112 A	10-06-1998
		CZ 9703775 A	17-06-1998
		HU 9702242 A	28-12-1998
		JP 10158291 A	16-06-1998
		PL 323407 A	08-06-1998

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/01499

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/66 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 47192 A (BIOSTORE NEW ZEALAND LIMITED) 18 December 1997 (1997-12-18) page 9, line 24-30; claims 5,34; examples 1,7	1-3, 5-12, 15-17
X	EP 0 324 227 A (RONCARI RAYMOND A) 19 July 1989 (1989-07-19) claims 4,6; tables 1,3	1-3, 5-12, 15-17
X	EP 0 652 012 A (NAITO ALBERT) 10 May 1995 (1995-05-10) abstract page 5, paragraph 2; claim 1; examples 13,18	1-3, 5-12, 15-17

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 1999	30/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gonzalez Ramon, N

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 845 475 A (HOECHST AG) 3 June 1998 (1998-06-03) page 4, line 1-35; table 1	1-17
A	JONES D. R. ET AL: "Review: The role of glycosyl-phosphatidylinositol in signal transduction" INT. J. BIOCHEM. CELL BIOL, vol. 30, 1998, pages 313-326, XP002115445 page 319 -page 320; figures 1,3	1-17
A	VARELA-NIETO I. ET AL: "Cell signalling by inositol phosphoglycans from different species" COMP. BIOCHEM. PHYSIOL, vol. 115B, no. 2, 1996, pages 223-241, XP002115446 abstract page 234, column 2; table 1	1-17
X,P	WILCOX R A ET AL: "New developments in the molecular pharmacology of the myo-inositol 1,4,5-trisphosphate receptor" TRENDS IN PHARMACOLOGICAL SCIENCES, vol. 19, no. 11, 1 November 1998 (1998-11-01), page 467-475 XP004142792 ISSN: 0165-6147 see 3D figure page 472; figure 1	1-17

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SJK/BP5767348		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01499	12/05/1999	29/06/1998
International Patent Classification (IPC) or national classification and IPC A61K31/66		
Applicant UNIVERSITY COLLEGE LONDON et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22/10/1999	13.04.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Simm, M.D. Telephone No. +49 89 2399 7411

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01499

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-23 as originally filed

Claims, No.:

1-17 as originally filed

Drawings, sheets:

1/8-8/8 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with r gard to nov lty, inv ntive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the report

D1: WO 97 47192 A (BIOSTORE NEW ZEALAND LIMITED) 18 December 1997 (1997-12-18)

D2: EP-A-0 324 227 (RONCARI RAYMOND A) 19 July 1989 (1989-07-19)

D3: EP-A-0 845 475 (HOECHST AG) 3 June 1998 (1998-06-03)

D4: JONES D. R. ET AL: 'Review: The role of glycosyl-phosphatidylinositol in signal transduction' INT. J. BIOCHEM. CELL BIOL, vol. 30, 1998, pages 313-326

D5: VARELA-NIETO I. ET AL: 'Cell signalling by inositol phosphoglycans from different species' COMP. BIOCHEM. PHYSIOL, vol. 115B, no. 2, 1996, pages 223-241

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The subject-matter of claims 1, 7, 8 and 15 appears to be novel and inventive in view of the prior art.

The present invention relates to the use of IPG for the preparation of a medicament for the treatment of ischaemic-reperfusion injury, a composition comprising IPG and ribose and a method of preserving an organ for transplantation with such a composition.

The description states that the acceleration of the restitution of myocardial adenine nucleotides by ribose has been already demonstrated (see page 2 in the description of the present application) but that the prior art approaches have not taken into account that the deficiency in ATP blocks the treatment with ribose. Therefore, an alternative approach to the problem of increasing the energy generating systems of the cell is required and the solution proposed is the use of IPG.
It appears that the technical problem itself and its solution are non-obvious in view of the prior art:

Compositions comprising IPG and Ribose are not disclosed in the prior art.

D1 presents compositions and methods for the preservation of living tissues but IPG is not mentioned therein.

D2 discloses compositions for increasing intracellular ATP but IPG is not mentioned therein.

D3 discloses the use of IPG for the treatment of Diabetes but the treatment of ischaemic-reperfusion injury is not mentioned.

The role of IPG in signal transduction is disclosed in D4 and D5 but none of these documents suggests the use of IPG for the treatment of ischaemic-reperfusion. Although a connexion between energy production and IPG through activation of pyruvate dehydrogenase can be found in these two documents (see tables), it appears that the skilled man in view of the prior art would have not been prompted to use IPG for the treatment of ischaemic-reperfusion injuries and thus, the present claims involve an inventive step.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

KIDDLE, Simon J.
MEWBURN ELLIS
York House
23 Kingsway
London WC2B 6HP
GRANDE BRETAGNE

17 APR 2000

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	13.04.2000
Applicant's or agent's file reference: SJK/BP5767348	**IMPORTANT NOTIFICATION**
International application No.: PCT/GB99/01499	International filing date *(day/month/year)*: 12/05/1999
Priority date *(day/month/year)*: 29/06/1998	
Applicant: UNIVERSITY COLLEGE LONDON et al.	

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer THORNTON, J Tel.+49 89 2399-8072

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